Identification of the Physical Modification Threshold of Dentin Induced by Neodymium and Holmium YAG Lasers Using Scanning Electron Microscopy

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IDENTIFICATION OF THE PHYSICAL MODIFICATION THRESHOLD OF DENTIN INDUCED BY NEODYMIUM AND HOLMIUM YAG LASERS USING SCANNING ELECTRON MICROSCOPY

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Abstract

Laser application to dentin has been advocated to modify the dentin substrate for restorative procedures. We examined the minimum energy density required to physically modify the dentin surface using 1.06 μm and 1.32 μm Neodymium:Yttrium-Aluminum-Garnet (Nd:YAG) and 2.10 μm Holmium-YAG (Ho:YAG) lasers. Three millimeter thick dentin sections from the middle occlusal third of crowns of third molars were used. To determine the effect of surface preparation, the sections were ground to 240, 320, 400, 600 grit or polished to 0.5 μm. Smear layer was removed using 0.5 M EDTA for 2 minutes. Five single pulse repetitions at each laser parameter were performed. Power (W) and energy per pulse (mJ/p) were increased for each wavelength until a physical modification occurred. The energy density (J/cm^2) was then held constant and the threshold was confirmed using 200, 320 and 550 μm diameter quartz contact probes. Scanning electron microscopy (SEM) was used to verify the physical modification of the dentin. The physical threshold remained constant for ground and polished surfaces. Similar surface modifications were found for the three wavelengths tested. The threshold modifications occurred at 207, 165, and 83 J/cm^2 for the 1.06 μm, 1.32 μm and 2.10 μm lasers, respectively. **For all emission wavelengths tested the physical threshold modification occurred at relatively low energy densities. These lasers show promise for surface modification of dentin.**

Key Words: Dentistry, dentin, lasers, scanning electron microscopy.

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Introduction

Lasers have been used in dentistry mainly for cutting and coagulating oral soft tissues (Pick and Pecaro 1987; Pogrel 1989; Silverman et al., 1988; White et al., 1991d). However, there is also considerable interest in the application of lasers on dental hard tissues. Continuous wave lasers have limited use for hard tissue primarily because of detrimental thermal effects from the relatively long interaction time with the substrate. Technological advances have occurred in lasers which allow microsecond pulses delivered through fiber-optic probes providing site specific delivery in the oral cavity. The family of Yttrium-Aluminum-Garnet (YAG) lasers appear to be promising for applications of this new technology, and the short interaction times permit use on dental hard tissues without the detrimental thermal effects seen with continuous wave lasers (Dederich et al., 1988, 1989; Leighty et al., 1991; White et al., 1992).

Dentin, the internal calcified tissue of teeth, is a difficult substrate for bonding of dental composite resin restorations. Bond strength of resins to dentin is low and microleakage is high, which leads to inadequate longevity of such restorations (Tao and Pashley 1988). Lasers show promise for modification of the dentin substrate to improve the bond strength of composite resin restorations (Cooper et al., 1988; White et al., 1991e). However, there has not been a systematic evaluation of the application of pulsed fiber-optic delivered lasers to induce modification of the dentin structures. This investigation was undertaken to determine the threshold for physical modification of dentin as a function of emission wavelength, energy density and surface texture of the dentin substrate.

Materials and Methods

Specimen Preparation

Freshly extracted third molars, caries and restoration free, were sterilized using gamma irradiation. Middle occlusal dentin sections were cut with a low speed saw and high concentration diamond wafering blade (Isomet, Buehler, Chicago, IL). The dentin sections were

cut to 3 ± 0.5 mm thickness. The dentin surface was further prepared by sanding with a strip grinder with pressure sensitive abrasive strips and polishing with diamond polishing pastes to 0.5 μm. Dentin sections were prepared to either 240, 320, 400, 600 grit or polished to 1/2 μm. All surfaces were treated with 0.5 M ethylene-diaminetetraacetic acid, EDTA (pH 7.4) for 2 minutes and washed with distilled water to remove smear layer produced during the cutting, grinding and polishing process. This process of specimen preparation produced dentin sections with varying surface textures from the coarse surface of 240 grit to that of the highly polished 0.5 μm surface (Figure 1 a-e). The dentin samples were kept under conditions of high humidity (moist) with 0.2% thymol solution to inhibit bacterial growth until tested.

Laser Modification

Three pulsed fiber-optic delivered YAG lasers were used. The emission wavelengths of these lasers were 1.06 μm and 1.32 μm produced by a Neodymium-YAG (Nd:YAG) laser and 2.10 μm produced by a Holmium-YAG (Ho:YAG) laser (Sunrise Technologies, Fremont, CA). The pulsed duration was 150 μs for the Nd:YAG lasers and 100 μs for the Ho:YAG laser. The laser energy was delivered to the dentin sections through circular quartz fiber-optic probes with diameters of either 200, 320, or 550 μm. The fiber-optic probes were placed in direct contact with the dentin surface. The fiber output energy per pulse (mJ/p) was varied in nine steps from 33 to 333 mJ/p and confirmed with an optical detector (Molectron, Medford, Oregon). The laser energy was delivered in single pulses to the prepared dentin sections starting with the lowest energy per pulse and increased until a photothermalacoustic event was seen and heard. This photothermalacoustic event indicated that a plasma had formed and that a physical modification may have occurred. The energy per pulse was then increased above this level in steps to the maximum of 333 mJ/p to confirm the threshold modification. Three emission wavelengths, nine energies per pulse, three fiber diameters, five surface textures and five repetitions at each energy level resulted in a broad exploration of laser parameters and potential laser dentin interactions in a significant data base (n = 2,025).

In addition to the single pulse interactions, multiple pulse interactions were made with the fiber-optic probe held stationary for one second at repetition rates of 5, 10, 15, and 20 Hz. Finally, to evaluate the effect of the lasers interaction across the surface of dentin, line exposures were made with the probe in contact and moved at a uniform rate across the dentin surface. The rates were determined for each probe diameter and repetition rate to allow for an overlap equal to one half the diameter of the probe as determined from equation 1.

Probe Rate [mm/sec]
$$= \text{(probe diameter/2 [mm])} * \text{(repetition rate [Hz])}$$
(1)

The desired probe rate was controlled using a me-

chanical testing machine (Instron Corp., Canton, MA). The range of laser parameters tested for the production of a line varied in powers from 0.3 to 3.0 W and frequencies of 5 to 20 Hz for probe diameters of 200, 320, and 550 μm.

Scanning Electron Microscopy (SEM)

The dentin sections were first examined visually for presence or absence of interaction. Given the large numbers of single pulse, multiple pulse and overlapping line laser/dentin interactions, the dentin specimens with obvious charring, well above the physical modification threshold, were eliminated. The remaining dentin sections were examined by SEM to determine the energy density in which the first physical modification of the dentin occurred. Wet SEM examination (ISI, Inc., model SX40A, Milpitas, CA) was performed at 15-30 kV with a Robinson scintillator backscatter detector at specimen chamber pressures of 0.12-0.5 torr to reduce charging according to the methods described by Marshall *et al.* (1989).

Results

The specific laser parameters of emission wavelength, energy per pulse, and energy density which produced a physical modification are given in Table 1. It was determined that the threshold energy per pulse to produce a surface physical modification on dentin for 1.06 μm was 167 mJ/p, for 1.32 μm was 133 mJ/p and for 2.10 μm was 67 mJ/p. The threshold energy per pulse decreased as emission wavelength increased. The physical modification of the dentin produced by one pulse at threshold for each emission wavelength appeared to be the same by SEM examination (Figures 2, 3 and 4). The modified dentin surface was melted and resolidified with partial closure of the dentinal tubules. The surface showed increased roughness with elimination of the striations from the tooth preparation procedure and the normal tubule structure of the dentin. The area immediately adjacent to the laser impact site appeared to be unaffected.

Varying the probe diameter allowed the threshold energy density identification for each emission wavelength. The energy density which produced the first surface physical modification using the 320 μm diameter probe for 1.06 μm was 207 J/cm^2, for 1.32 μm was 165 J.cm^2 and for 2.10 μm was 83 J/cm^2. When energy densities were lower than the threshold there was no physical change in the dentin surface seen by SEM examination. When energy densities were higher than the threshold, the dentin surface was altered physically, independent of probe diameter. When the probe diameter was 200 μm, the energy density at threshold was 213 J/cm^2 for the 1.06 μm emission wavelength Nd:YAG laser. This was the same energy density which caused a physical modification of dentin using the 320 μm diameter probe. The energy per pulse, however, was lower for the 200 μm probe (67 mJ/p) compared to the 320 μm probe (167 mJ/p). When probe diameter was increased, the energy per pulse required to physically
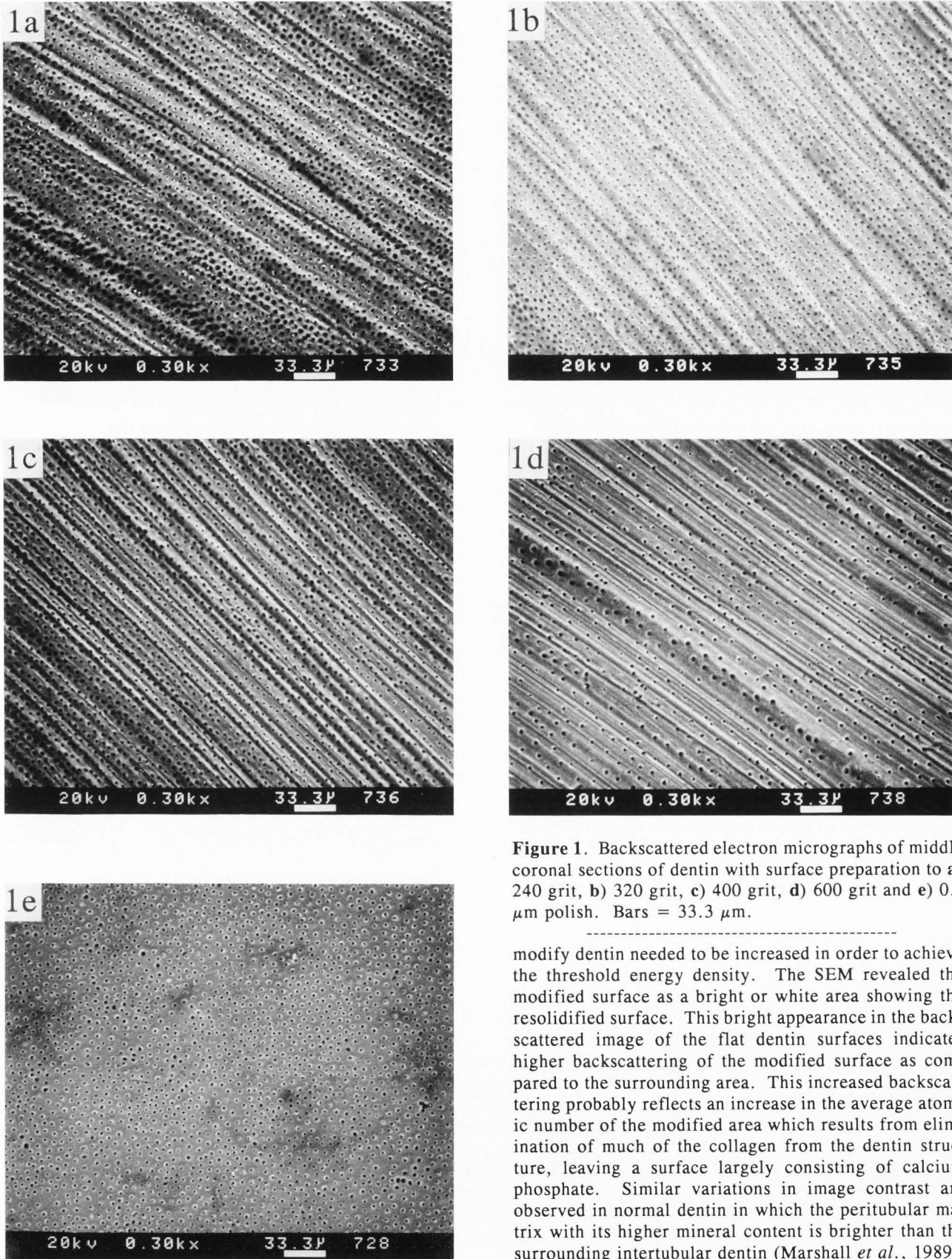
Figure 1. Backscattered electron micrographs of middle coronal sections of dentin with surface preparation to **a)** 240 grit, **b)** 320 grit, **c)** 400 grit, **d)** 600 grit and **e)** 0.5 μm polish. Bars = 33.3 μm.

modify dentin needed to be increased in order to achieve the threshold energy density. The SEM revealed the modified surface as a bright or white area showing the resolidified surface. This bright appearance in the backscattered image of the flat dentin surfaces indicates higher backscattering of the modified surface as compared to the surrounding area. This increased backscattering probably reflects an increase in the average atomic number of the modified area which results from elimination of much of the collagen from the dentin structure, leaving a surface largely consisting of calcium phosphate. Similar variations in image contrast are observed in normal dentin in which the peritubular matrix with its higher mineral content is brighter than the surrounding intertubular dentin (Marshall *et al.*, 1989).

Table 1. Laser parameters tested where Ed = energy density (J/cm^2) for each probe diameter of 200, 320, and 550 μm. Bold type indicates laser parameter where physical modification threshold of dentin was identified.

Wavelength (μm)	Energy Per Pulse (mJ/p)	ED (J/cm^2) for lasers of 200 μm	320 μm	550μm
106	33	105	41	14
	67	**213**	83	28
	100	318	124	42
	133	423	165	56
	167	531	**207**	70
	200	636	249	84
	233	741	290	98
	267	850	332	112
	333	1060	414	140
132	33	105	41	14
	67	**213**	83	28
	100	318	124	42
	133	423	**165**	56
	167	531	207	70
	200	636	249	84
	233	741	290	98
	267	850	332	112
	333	1060	414	**140**
210	33	**105**	41	14
	67	213	**83**	28
	100	318	124	42
	133	423	165	56
	167	531	207	70
	200	636	249	**84**
	233	741	290	98
	267	850	332	112

SEM examination of the lines showed similar dentin physical modification (Figure 5). In areas of the line where the pulses were overlapped there was a slightly increased surface modification. The surface modification at threshold by single pulses and in the lines was consistent and not dependent on surface preparation. SEM examination of cross-sections through the lines on dentin determined the physical modification to be limited to less than 50 μm in depth (Figure 6). The area immediately under and adjacent to the laser impact site was unaffected, with normal dentinal tubules, peritubular and intertubular structure.

Visually the surfaces modified by the lasers appeared different. At the threshold, the Nd:YAG (1.06 μm and 1.32 μm) modified dentin surfaces were darkened tan to brown. The Ho:YAG laser, however, produced surfaces which were white in appearance. This may indicate an improvement in the combustion of collagen without carbonization. Above the physical threshold, the dentin surfaces were either tan, brown or black. SEM analysis below the physical threshold showed no change in dentin for all emission wavelengths tested. At

Figure 2. Morphologic appearance of the physical modification (m) of dentin at threshold from single pulse of Nd:YAG (λ = 1.06 μm) laser, energy per pulse = 167 mJ/p, energy density = 207 J/cm^2, using a 320 μm contact fiber-optic probe. Bar = 33.3 μm.

Figure 3. Physical modification threshold of dentin after one pulse of Nd:YAG (λ = 1.32 μm), energy per pulse = 133 mJ/p, energy density = 165 J/cm^2 using a 320 μm contact fiber-optic probe. Arrow indicates area of melted and resoidified dentin. Bar = 33.3 μm.

Figure 4. Physical modification (m) threshold of dentin after one pulse of Ho:YAG (λ = 2.10 μm), energy per pulse = 67mJ/p, energy density = 83 J/cm^2 using a 320 μm contact fiber-optic probe. Bar = 33.3 μm.

Figure 5. Linear surface modification of dentin produced by laser exposure, right to left increasing laser power. I_1 to I_6 indicate linear melting and resolidification. Bar = 667 μm.

Figure 6. Cross-section through a linear modification of dentin showing the laser alteration of dentin (arrow) to be limited to the surface with no physical alteration below approximately 20 μm. Bar = 40 μm.

Figure 7. Ablation of dentin from no surface modification (d) to physical modification threshold (m) and beyond physical modification threshold with ablation crater (ac). Bar = 500 μm.

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the physical threshold, the dentin was uniformly modified with no cracking, but above the threshold the dentin was ablated and crater formation was present (Figure 7). This was particularly evident in the multiple pulse laser exposures on dentin. As the number of pulses and the energy per pulse were increased the size and depth of the crater that was formed similarly appeared to increase. The most damaging interactions on the dentin were caused by high energies per pulse, long exposure times and high powers and repetition rates. The detrimental interactions consisted of surface cracking, ablation of dentin and crater formation.

Discussion

This investigation determined the physical modification threshold of dentin from the interaction of pulsed fiber-optic 1.06 μm and 1.32 μm Nd:YAG and 2.10 μm Ho:YAG lasers with the dentin surface. The physical threshold was defined as the first interaction that occurred causing an observable change in the dentin structure. The identification of a physical threshold for dentin is critical in the understanding of specific interactions of laser parameters such as wavelength, energy per pulse and energy density on dentin. Below the physical threshold, chemical changes of the substrate may occur, which may be seen first as changes in the collagen matrix of dentin. Collagen would be expected to be affected first as it is organic and denatures at low temperatures. Thus collagen would be altered and may even
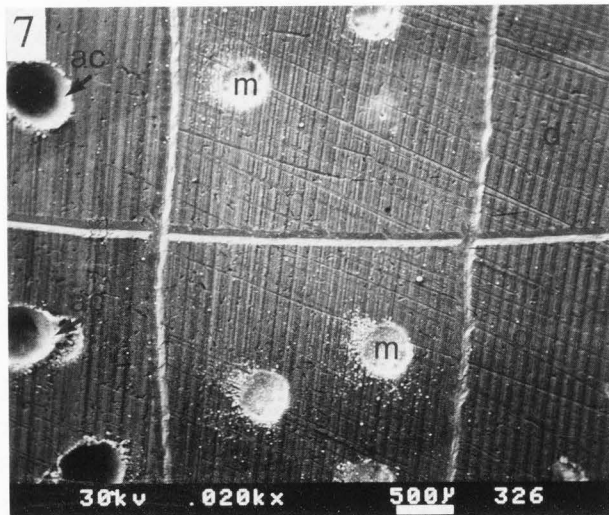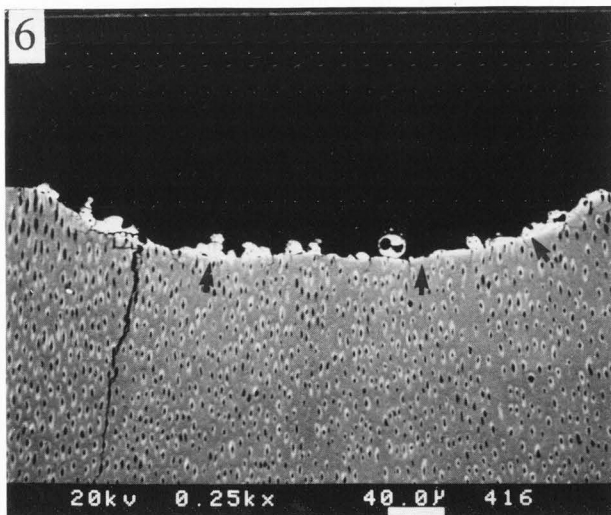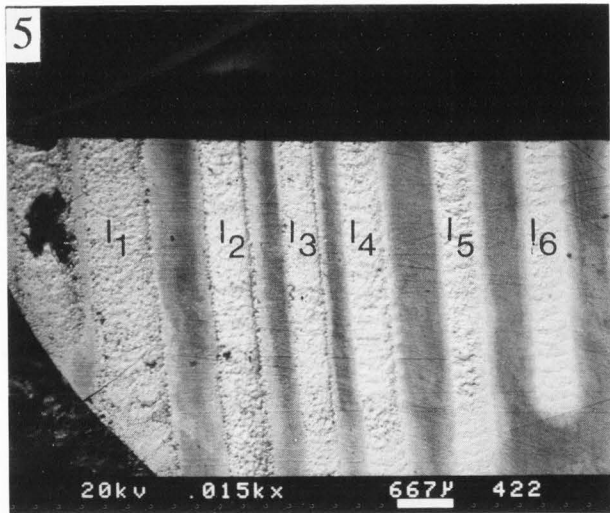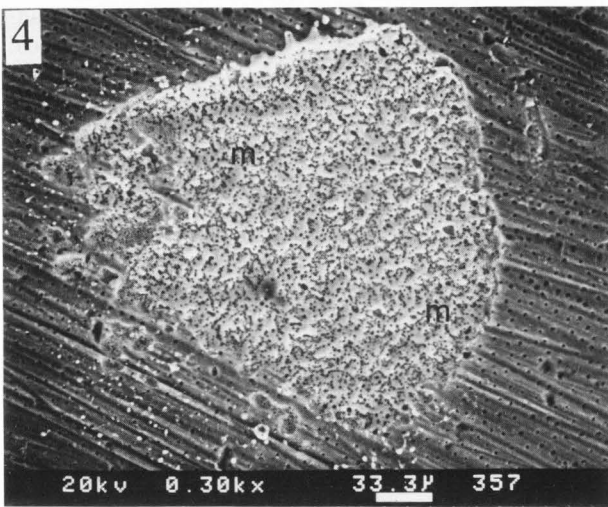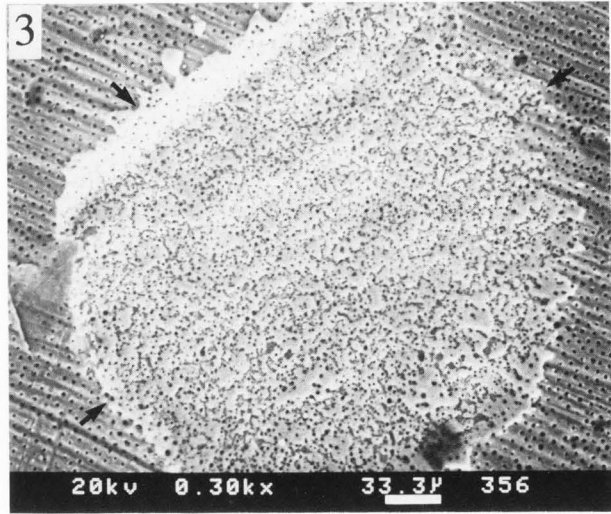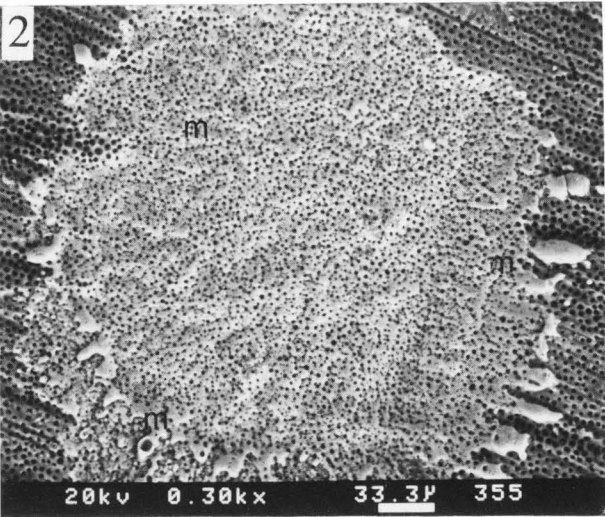
char at temperatures well below temperatures which would induce significant chemical changes in the inorganic matrix of dentin (White *et al.*, 1991c). In order to determine if these chemical changes have occurred, other analytical techniques such as FTIR, EDS and X-ray diffraction must be employed. At the physical threshold, the laser interaction may induce both a chemical and physical change which must be fully characterized utilizing standard mechanical tests such as microhardness with further chemical analysis. A variety of techniques has been used successfully to study laser induced modification of enamel and can also be applied to the laser interactions with dentin (Nelson *et al.*, 1986; Featherstone and Nelson 1987; Fox *et al.*, 1992).

The specific morphologic appearance at threshold is a property of the dentin substrate and its interaction with the specific laser parameters. Because each emission wavelength at threshold caused the same morphologic appearance, the threshold identified is a property of the dentin. The differences in energy density for each wavelength needed to cause a modification of dentin indicates that the absorbance of dentin at each wavelength is different. The absorbance of dentin increases as the wavelength increases in the range of emission wavelengths tested.

The laser treatments created various color changes in the dentin which suggests additional study is needed of the optical properties of laser modified dentin. The colors probably are associated with various combustion products from burning and charring of the collagen. The nature of the products and their amounts most likely are dependent on the laser parameters, so that variations could occur in the products themselves and interactions via the photothermalacoustic event, leading to varying amounts of each product deposited in the surrounding dentin. Thus darker discolorations might indicate a large zone of incomplete combustion, while a white zone could indicate either complete combustion or expulsion of combustion products from the modified dentin surface.

In this investigation, when laser parameters were well above the physical threshold, ablation of dentin occurred. This resulted in crater formation with burning and charring similar to that seen with the use of other wavelengths such as the carbon dioxide laser (Leighty *et al.*, 1991; Dederich *et al.*, 1988, 1989) and continuous wave free beam Nd:YAG lasers (Dederich *et al.*, 1984). Because of the short interaction times and high peak powers, the microsecond pulsed lasers investigated in this study had minimal thermal effects to the underlying structures. Furthermore, charring should be minimized at parameters causing ablation. This explains the observation that areas immediately adjacent and below the laser modified region appear to retain all the features of the normal dentin structure.

Determination of the physical threshold is important for defining parameters to meet specific dental treatment objectives which involve alterations of the dentin surface. Modifications of the dentin surface are current-ly under study for both therapeutic and restorative procedures. Sealing or closing open tubules may be of benefit in reducing sensitivity and pain as well as increasing resistance to caries. The surface modifications seen in this study at the physical threshold have been shown to increase microhardness (White *et al.*, 1991b). The modified dentin also could increase micromechanical retention for composite restorations (Cooper *et al.*, 1988). Our initial investigations have determined that increased bond strengths (White *et al.*, 1991f) do occur, but the presence of a weakened char layer may interfere with the bonding mechanism.

The partial closure of the dentin tubules should alter fluid flow through the dentin, although a completely impermeable layer has not been demonstrated. In clinical use, coagulation of the proteins contained in the dentinal fluid is also expected to reduce the fluid flow (Goodis *et al.*, 1992). We would expect that physical modification of the dentin surface combined with coagulation of the protein contained in the dentinal fluid would decrease permeability and may lead to methods to decrease root surface sensitivity. Further studies addressing the acid resistance, microleakage and clinical benefits of laser modified dentin are necessary.

The dentin surface changes with these pulsed fiber-optic delivered lasers were consistent over the range of surface preparation conditions tested. This indicates that it is the absorption of laser energy by the dentin substrate that allows the interaction to occur. The addition of highly absorbing dyes or pigments on the dentin surface would be likely to decrease the physical threshold values. The physical threshold for such absorbance initiators and carious dentin, which is often pigmented, are currently under investigation. Since the objective of this study was to modify dentin surfaces, the laser parameters used were lower than laser parameters used for cutting or drilling dentin through ablation. Further analysis of ablation parameters and rates is therefore needed. The laser energy delivered to the dentin results in a photothermalacoustic interaction, which is largely confined to the surface. Cross-sections of the dentin displayed no alteration of the underlying dentin, indicating that the temperature rise may be superficial and may not cause adverse pulpal reactions. The surface modifications defined in this study combined with previous pulpal and thermal penetration depth studies (White *et al.*, 1991a; Goodis and White 1992; White *et al.*, 1992) indicate that these microsecond fiber-optic delivered lasers can create specific modification of the dentin surface without detrimental effects to adjacent tissue.

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Discussion with Reviewers

D.G. Nelson: Please describe more fully what a "photothermalacoustic event" is? Is it possible to have a "photoemission event" without an "acoustic event"?
Authors: The photothermalacoustic event described in this paper refers to the laser emission being absorbed by the dentin surface (photo), the rapid heating of the dentin surface (thermal) and the shockwave generated as the surface rapidly boils (acoustic). Because these lasers have microsecond pulse durations with peak powers in the kilowatt range there is a photothermalacoustic event. It is possible to have a photoemission event (i.e., heating of the surface) without a photoacoustic event only if the lasers were very long pulse durations or continuous wave with low peak powers. Slow heating of the surface (a hot tip effect of the laser) would allow for a photoemission without an acoustic event but this does not happen with the high peak power short pulse duration lasers used in this study.

D.G. Nelson: In understanding why the observed threshold modification occurred at different energy densities for the three different wavelengths tested, it would be helpful to have reported the infrared (IR) spectra of dentin, collagen and water. Would the authors like to provide this data and discuss which of these components is responsible for the variation in absorbance with wavelength?
Authors: The IR data available for collagen and water use transmission Fourier Transform Infrared Spectroscopy (FTIR) in solution. This data is limited, as dentin is anisotropic solid consisting of mineral, collagen and

water. We have developed specular reflectance FTIR and UV/VIS/NIR spectroscopy techniques and are currently investigating dentin surfaces to gain a better understanding of the optical properties of dentin in this region of the electromagnetic spectrum.

D.G. Nelson: Cross-sections of ablated regions showed a thin hypermineralized zone approximately 5 μm thick immediately beneath the ablated surface. Is this the thickness of the melt zone? At threshold the craters were several tens of microns deep, surely threshold would be at the start of ablation? Below threshold was there any evidence of surface melts or thin hypermineralized zones?

Authors: Cross-sections of the ablated regions did have a thin melted zone which is the resolidified surface after the ablation. The reviewers approximations of the craters depth are correct in that tens of micrometers are removed by a single pulse at threshold and the resulting melted and resolidified surface appears as a hypermineralized zone. We are currently trying to maximize this hypermineralized zone and will investigate its resistance to demineralization. Below threshold, there was no evidence of surface melting or hypermineralized zones primarily because the pulse duration of the lasers was so short that there was not heating of the surface to cause melting.

D.G. Nelson: The authors mention the use of highly absorbing dyes or pigments to reduce physical threshold levels. Does this not assume that there is an efficient thermal energy transfer from the dye to the surrounding dentin, otherwise the dye would simply be ablated preferentially from the surface?

Authors: The assumption is that there is thermal energy transfer from the dye to the dentin. It is unknown if this is an efficient process. The pigments are absorbed by the dentin and therefore, they alter the surface of dentin changing the optical properties of the dentin. The pigments used are thin films which do not whip off from the dentin surface and therefore, the dentin must be ablated. It is possible that pigments, that are not absorbed by the dentin, could be preferentially ablated, by very short pulse duration lasers, leaving the surface unaltered but it is unlikely in the range of laser parameters studied.

P.P. Phakey: How do the threshold energies for the Nd:YAG and Ho:YAG lasers compare with the CO_2 laser?

Authors: It is generally assumed that the carbon dioxide laser has a **very** high absorbance for water and therefore, would have an equally high absorbance for oral tissues. We did not investigate the physical threshold energies for the carbon dioxide laser on dentin and are unaware of any scientific publication which specifically determined the physical threshold energies for the carbon dioxide laser on dentin.

P.P. Phakey: Was the spot size measured on the speci-

men after irradiation or was it the contact area with the fiber? For the energy density calculations, was the energy measured on the specimen or was it the value given by the manufacturer?

Authors: The spot size was determined by measurement of the diameter of the fiber. The fiber was then placed in direct contact with the dentin specimen. The ablation sites on the dentin actually appeared slightly larger ($\leq$ 10%) than the fiber diameter which may be due to the volume of the plasma generated and the escape of the ablated material around the sides of the fiber during the ablation process. The energy density calculations were made using the measured diameter of the fiber and the energy per pulse exiting the fiber tip as measured by an optical detector. The lasers supplied by the manufacturer were accurate and the displayed laser parameters were confirmed by direct measurement of the output energy at the fiber tip.

C.M. Cobb: Were the optical fibers positioned at right angles to the surface (photographs would indicate they were)?

Authors: Yes, the optical fibers were placed in direct contact with the dentin surface and held so that the fiber was at right angles to the surface.

T.D. Myers: The authors state that the threshold energy per pulse decreased as emission wavelength increased. Can this be taken as a general rule for the entire electromagnetic spectrum?

Authors: No, the optical properties of dentin (reflectance, transmittance and absorbance) are not constant through the electromagnetic spectrum and therefore, the threshold energy per pulse must be determined for each emission wavelength. Additionally, the laser parameters of pulse duration and peak power can be manipulated to overcome low absorbance so direct measurement for each laser system is required to determine the physical threshold and surface effects on dentin.

T.D. Myers: Was there evidence of creation of a smear layer at or adjacent to the laser modified surface?

Authors: The area immediately adjacent to the laser impact site appeared to be unaffected. Because there was no debris as seen on the surface of the dentin, there was no smear layer created and the it appears as the ablated material was completely volatilized and ejected from the surface. Therefore, laser interaction with dentin does not create a smear layer. If smear layer were present on the surface, we would expect that it would be removed readily at or above the physical threshold because the smear layer is only a few microns in thickness.